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The Immunoglobulin Class Switch: Beyond "Accessibility"

Minireview

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Induction of germline constant heavy (C_H) gene transcription by cytokines, B cell activators, or both is a key event for targeting the C_H gene for subsequent switch rearrangement. An increase in transcriptional activity may confer to the C_H locus a state of enhanced accessibility for the binding of additional factors important for mediating the switching event. This regulatory paradigm is known as the "accessibility" model of immunoglobulin class switching. Here we discuss recent studies demonstrating the ability of interleukin-4 (IL-4), IL-5, IL-10, interferon-γ (IFNγ), membrane immunoglobulin–mediated activation, and the transcription factor, p50/NF-κB to mediate distinct and profound changes in immunoglobulin class switching in the absence of corresponding alterations in germline C_H RNA expression. These studies suggest novel modes of regulation of the immunoglobulin class switch that may involve levels of accessibility distinct from transcriptional activation or alterations in the recombination machinery itself (or both).

The immunoglobulin class switch is a process that is critical for the generation of functional diversity of a humoral immune response (for review see Coffman et al., 1993; Harriman et al., 1993; Snapper and Finkelman, 1993; Lorenz and Radbruch, 1996; Stavnezer, 1996). At the cellular level, switching is manifested by the transition from B cells expressing membrane immunoglobulin M (mIgM), IgD, or both to those expressing IgE, IgA, or one of four IgG subclasses. Immunoglobulin classes are encoded by C_H genes aligned in tandem, 3′ to a rearranged VDJ gene that encodes for antigen specificity. 5′ to each C_H gene, except $C_H\delta$, are blocks of tandemly repetitious DNA sequences, termed switch (S) regions. At the molecular level, the predominant mode of immunoglobulin class switching comprises a recombination event that includes looping out and deletion of all C_H genes 5′ to the C_H gene that is to be expressed (Iwasato et al., 1990; Matsuoka et al., 1990; von-Schwedler et al., 1990) (Figure 1). In a B cell that is initially $mIgM^+$, this recombination event occurs between Sμ and the S region of the downstream C_H gene. Sequential switching may also occur through the deletion mechanism (Mills et al., 1992, 1995; Siebenkotten et al., 1992; Mandler et al., 1993a).

Germline C_H RNA

Prior to switch rearrangement, populations of activated B cells typically express one or more characteristic RNA species encoded by different C_H genes in the germline (Stavnezer-Nordgren and Sirlin, 1986; Yancopoulos et al., 1986). Germline C_H RNAs are spliced products of distinct I exons, located 5′ to every S region, and the immediate 3′ C_H gene (Figure 1). Transcription initiates within a promoter 5′ to the I exon, proceeds through the S region, and terminates at the 3′ end of the C_H gene. Splicing out of the S region creates the final germline C_H RNA (i.e., 5′ I_HC_H 3′) (Lutzker and Alt, 1988; Gaff and Gerondakis, 1990; Gerondakis, 1990; Lebman et al., 1990; Radcliffe et al., 1990; Rothman et al., 1990). Multiple chimeric germline C_H transcripts ($I\mu C\epsilon$, $I\epsilon C\mu$, $I\mu C\gamma 4$, $I\gamma C\mu$, $I\gamma C\epsilon$, $I\epsilon C\gamma$, and $I\gamma 4C\alpha 1$) have also been demonstrated in IgD^+ human B cells stimulated by IL-4 alone, in the apparent absence of either genomic switch recombinations or their circular by-products (Fujieda et al., 1996). The authors interpret their findings as evidence for *trans*-splicing of unrearranged germline immunoglobulin RNA transcripts. Additionally, spliced hybrid $I\mu C\gamma$ transcripts can form subsequent to switch recombination through processing of a single RNA transcript containing the juxtaposed Iμ and Cγ regions and accumulate along with the respectively mature Cγ RNA (Li et al., 1994). These data are consistent with the model that simultaneous germline expression of both 5′ and 3′ C_H genes (Cμ and Cγ) is involved in targeting their subsequent switch recombination. Germline C_H RNAs lack VDJ-encoded sequence and hence cannot, by themselves, direct synthesis of intact immunoglobulin molecules. One group has shown that a germline C_H transcript has the potential to encode a truncated Cμ protein in vitro (Bachl et al., 1996). However, the potential existence of germline C_H RNA–encoded, truncated C_H proteins in vivo remains controversial, as most I exons contain translation stop codons in each reading frame (Lennon and Perry, 1985). Upon switch rearrangement, the I and S regions are deleted and the relevant C_H gene is brought into proximity with the VDJ gene (Figure 1). In this position it encodes a productive VDJ-C_H RNA for synthesis of an intact immunoglobulin molecule expressing the same antigen specificity but a new immunoglobulin class.

The "Accessibility" Model of Immunoglobulin Class Switching

The immunoglobulin class switch is influenced in both a positive and negative manner by a number of cytokines and B cell activators. The mechanism for this appears to lie in part in the ability of cytokines and activators to regulate transcription selectively, which initiates upstream of each of the I exons. This effect is manifested by changes in the steady-state levels of the different germline C_H RNA after activation. The selectivity of this process lies in the presence of unique sequences 5′ to each I exon for binding of a series of regulatory proteins whose expression within the B cell may be influenced by the activation conditions. However, the function of the I exon itself is unknown. Based upon precedents in other cellular systems, it has been proposed that the

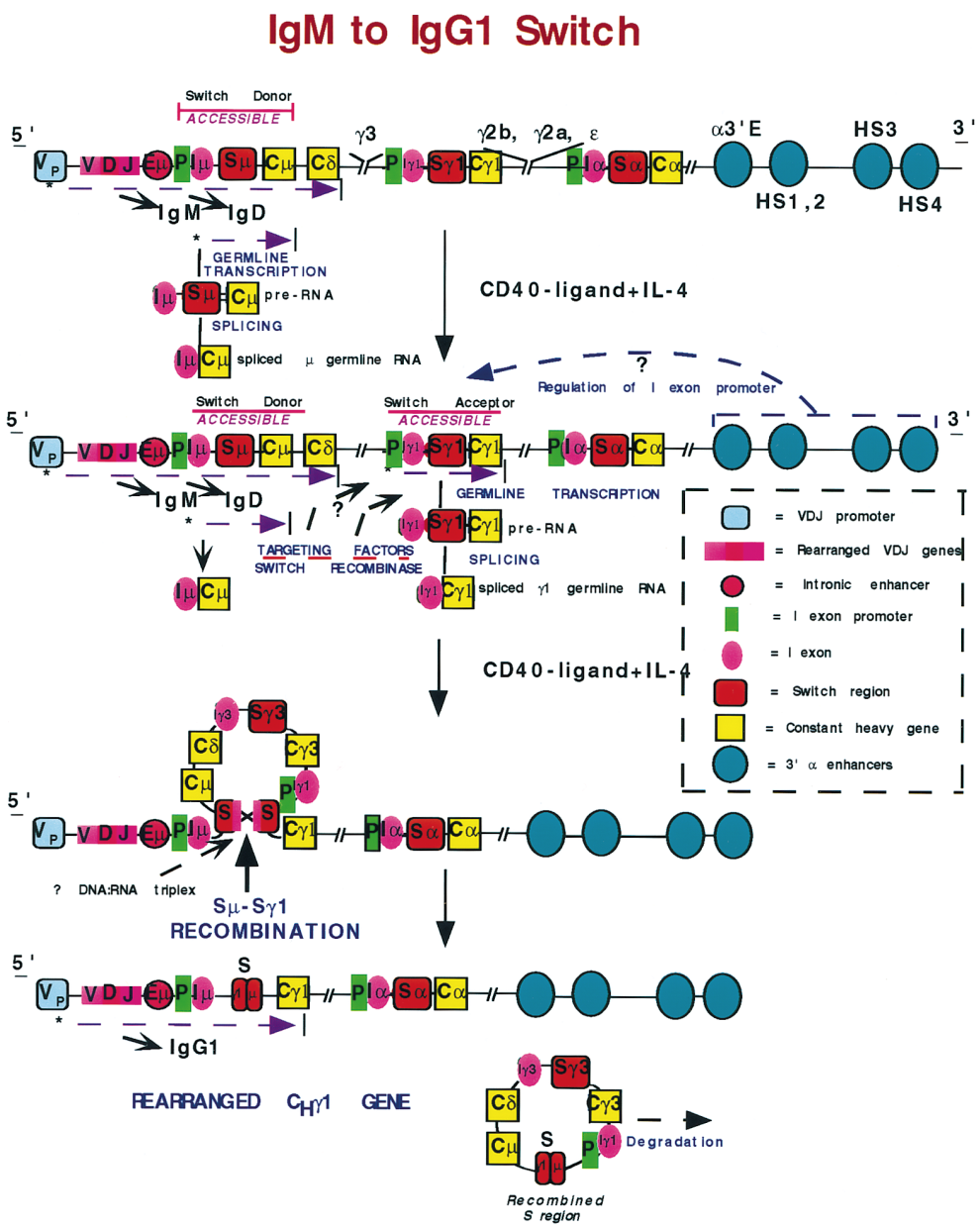

Figure 1. Schematic of the Immunoglobulin Class Switch (e.g., IgM to IgG1)

events that initiate germline C_H transcription also confer a level of accessibility to the C_H locus for the binding of additional regulatory elements that participate in switch recombination (i.e., the accessibility model of immunoglobulin class switching; Stavnezer-Nordgren and Sirlin, 1986; Yancopoulos et al., 1986).

Analyses of switch-recombinase substrates, either chromosomally integrated or in an episomal state, have engendered further support for the accessibility model by demonstrating a requirement for substrates to be transcriptionally active in order to undergo switch rearrangement (Ott et al., 1987; Ott and Marcu, 1989; Leung and Maizels, 1992, 1994; Ballantyne et al., 1995; Daniels and Lieber, 1995a). Further, this transcriptional requirement is DNA-strand specific (Reaban and Griffin, 1990; Daniels and Lieber, 1995a, 1995b). Thus, a cellular switch-recombinase activity appears to operate on appropriate switch sequences even when they are present outside of their natural chromosomal contexts. The S sequence–specific-recombinase activity acting on chromosomally integrated switch substrates also appears to be B-cell specific (Ott et al., 1987; Ott and Marcu, 1989; Leung and Maizels, 1992, 1994; Ballantyne et al., 1995, 1997; Daniels and Lieber, 1995a). One study demonstrated that Sγ and Sα substrate sequences could recombine with Sμ in the same pre-B cell line, implying that a common recombinase may exist for all S sequences (Lepse et al., 1994). In contrast, replicating extrachromosomal switch substrates (unlike their natural genomic counterparts or integrated, proviral substrates;

Ott et al., 1987; Ott and Marcu, 1989; Ballantyne et al., 1995, 1997) undergo significant recombination in inappropriate cell types and can also produce non-S sequence recombination products (Leung and Maizels, 1992, 1994; Daniels and Lieber, 1995a), implying that constraints of substrate specificity are more relaxed on such replicating episomal, recombination targets. Recent work with single copy, chromosomally integrated retroviral substrates indicates that recombinase activity is not only B-cell specific, but B cell–stage specific (restricted to cell lines representing late stage pre-B and mature B cells; Ballantyne et al., 1997). Furthermore, the recombinase activity was found to act in a stochastic fashion upon constitutively accessible (i.e., transcribed) S region targets, in that switch recombinations were found to be chance events likely occurring with similar probability from one cell generation to the next (Ballantyne et al., 1997). Whether the switch-recombinase activity itself is subject to regulation by various B cell stimuli remains an open question.

Two recent reports employing homologous gene targeting in mice have now shown that I regions strongly target class switching to specific S regions beyond their ability simply to induce transcription (Bottaro et al., 1994; Lorenz et al., 1995). In one of these studies it was proposed that the spliced I_HC_H RNA may also be required (Lorenz et al., 1995). Bottaro et al. (1994) have shown that when the natural lipopolysaccharide (LPS)/IL-4-inducible Iε promoter and exon is replaced by an LPS-inducible Eμ enhancer/V_H promoter, switching to IgE is inhibited, in *cis*, by 10- to 100-fold in spite of substantial transcription through the Sε region of the targeted allele. Therefore, optimal switching requires an intact I region or I region promoter in *cis* (or both) beyond their contributions to S region transcription. Lorenz, Jung, and Radbruch went on to report that the Iγ1 exon sequences encompassing the splice donor signal were necessary and sufficient for normal levels of switching to IgG1, implying that the spliced germline transcript or the spliceosome complex was in some way required for subsequent switch recombination. However, in this latter study the mutant γ1 locus that retained the I exon splice donor possessed much higher transcriptional activity than the switching-defective, mutant γ1 locus which lacked the same splice donor signal. Therefore, a more direct role of the processed germline transcript or its splicing intermediate in subsequent switch-recombination remains controversial.

It has been suggested that transcription of S sequences prior to their recombination may produce an RNA:DNA triplex switch recombination substrate. Reaban and Griffin first demonstrated that the supercoiled state of Sα polypurine repeats is lost upon their transcription, but that the configuration of these repeats remains stable by virtue of DNA strand–specific RNA:DNA hybrid formation. It was suggested that such stabilized, triplex strands of RNA:DNA conformers would exist just prior to switch recombination (Reaban and Griffin, 1990). In vitro transcription of Sγ sequences has also recently been shown to produce strand-specific RNA:DNA hybrid structures, providing additional support for the idea of an RNA:DNA intermediate culminating in switch recombination (Daniels and Lieber, 1995b). Another group has independently hypothesized that such S sequence–specific RNA:DNA hybrids could indirectly enhance the overall recombinagenic activity of S regions by specifically facilitating the formation of nuclease-sensitive, short stem loop structures among the self-complementary S region tandem repeats (Baar et al., 1996).

Over and above control of germline C_H RNA expression by a promoter 5′ to the I exon, IgH locus transcriptional control in more general terms is orchestrated through the actions of an intronic enhancer (Eμ), located between J_H and $C_H\mu$, and a developmentally regulated enhancer complex located 3′ of Cα (3′E) and consisting of four enhancers: α3′E, 3′E-HS1,2, 3′E-HS3, and 3′E-HS4 (Dariavach et al., 1991; Lieberson et al., 1991; Matthias and Baltimore, 1993; Madisen and Groudine, 1994; Michaelson et al., 1995). Eμ is implicated, through its early transcriptional activation of DJ-$C_H\mu$ sequences, in making Sμ accessible for subsequent recombination with downstream S regions (Gu et al., 1993). One study employing episomal S sequence substrates observed that Eμ was even capable of stimulating switch recombination above its effects on transcriptional activity (Leung and Maizels, 1994). The evidence for a role of the 3′E region in germline C_H transcription comes from homologous gene targeting in mice. Here, replacement of 3′E-HS1,2 by a heterologous neomycin gene expression cassette strongly repressed most germline C_H RNAs (Cogne et al., 1994). As anticipated from the accessibility model, this was associated with a marked reduction in switch rearrangements of the corresponding C_H genes. However, the foreign *neo* expression cassette may have contributed to the latter effects by disturbing, perhaps through enhancer competition, the function of the other enhancers of the 3′E region or other yet to be defined regulatory elements. A study demonstrating that the two most distal enhancers of the 3′E may impart additional properties to the more proximal enhancers is consistent with the notion that the 3′E acts as a locus control region (Madisen and Groudine, 1994). The 3′E region may selectively regulate transcription of germline C_H RNAs, but the nature of the cross-talk between individual I exon–associated promoters and specific 3′E enhancers remains to be delineated.

An extensive literature lends strong support for the accessibility model of immunoglobulin class switching. This is evidenced by numerous observations that switching to a particular immunoglobulin class is always preceded by the appearance of germline C_H RNA corresponding to that class (Coffman et al., 1993; Harriman et al., 1993; Snapper and Finkelman, 1993; Lorenz and Radbruch, 1996; Stavnezer, 1996). Further, several studies have demonstrated a direct correlation between cytokine-mediated induction of germline C_H RNA and enhanced transcriptional rate at the relevant C_H locus (Rothman et al., 1991; Shockett and Stavnezer, 1991; Lorenz et al., 1995), although a single report challenged this notion (Lebman et al., 1994). Although germline C_H RNA can be induced in a resting B cell, such cells will not undergo switch rearrangement unless they have also entered the cell cycle (Severinson et al., 1982; Kenter and Watson, 1987; Lundgren et al., 1995). Several reports have suggested a direct role for DNA synthesis as

Table 1. Changes in Immunoglobulin Class Switching Independent of Alteration in Germline C_H RNA Expression

"Switch Factor"	Immunoglobulin Isotype	Activation Conditions	Reference
IL-4	↑ IgA	CH12F3 B lymphoma +/− CD40 ligand and/or TGFβ	Nakamura et al., 1996
IL-5	↑ IgG1	Anti-Ig-dex + IL-4	Mandler et al., 1993
	↑ IgG1 + IgE	Sepharose–anti-Ig + LPS	Purkerson and Isakson, 1992
IL-10	↑ IgG3	LPS	Shparago et al., 1996
	↓ IgA	LPS + IL-4 + IL-5 + anti-Ig-dex + TGFβ	Shparago et al., 1996
IFNγ	↓ IgG3	LPS	Severinson et al., 1990 Zelazowski et al., 1995
Anti-Ig-dex	↓ IgG1	LPS + IL-4	Zelazowski et al., 1995
Pharmacologic Agent			
DSCG	↓ IgE (human)	Anti-CD40 + IL-4	Loh et al., 1994
Transcription Factor			
p50/NF-κB knockout	↓ IgA	LPS + IL-4 + IL-5 + anti-Ig-dex + TGFβ	Snapper et al., 1996

Unless otherwise indicated, these studies utilized normal murine B cells in vitro. DSCG, disodium cromoglycate.

an element or component of the switch rearrangement process (Dunnick et al., 1989; Dunnick and Stavnezer, 1990) and even germline C_H RNA expression (Lundgren et al., 1995), but the molecular mechanisms remain to be elucidated.

Switch Rearrangement May Be Regulated Independently from Alterations in Germline C_H RNA Expression: An Expanded View of Immunoglobulin Class Switch Regulation

Although DNA synthesis and germline C_H gene transcription appear to be necessary for switch rearrangement to occur, a large body of evidence, utilizing normal and transformed murine B cells, now strongly suggests that these two processes are not sufficient. Indeed, a series of regulated events in cycling B cells has been observed that leads to striking changes in switch rearrangement without corresponding changes in germline C_H RNA expression. These observations, discussed in detail below, imply that in addition to germline C_H transcription, additional events under the control of a number of cytokines, B cell activators, and transcription factors (summarized in Table 1) or other targeting factors may also be central to controlling the immunoglobulin class switch. The identification of these regulated components represents a key challenge for investigators in the field.

In most of the studies discussed below, three methodologies were utilized to assess immunoglobulin class switching: flow cytometric analysis of the percentage of B cells expressing various mIg isotypes consequent to in vitro activation; digestion circularization–polymerase chain reaction (DC–PCR) for quantitating specific switch rearrangement events at the DNA level (Chu et al., 1992); and semi-quantitative reverse transcription–PCR for comparative assessment of germline C_H RNA expression.

IL-4

IL-4 is a switch factor for the IgG1 (Isakson et al., 1982; Severinson et al., 1982) and IgE isotypes (Coffman and Carty, 1986), and this property is associated with IL-4 induction of germline $C_H\gamma1$ and $C_H\epsilon$ RNA (Stavnezer et al., 1988; Rothman et al., 1988). A role for IL-4 in switching to IgA was recently assessed using a B cell lymphoma model. CH12F3, a subclone of the CH12.LX B cell lymphoma which constitutively expresses germline $C_H\alpha$ RNA, is induced to switch at a high frequency (~50%) in response to the combination of CD40 ligand, IL-4, and transforming growth factor β (TGFβ) (Nakamura et al., 1996). IL-4 alone enhances switching to IgA (up to 12%) in CH12F3 cells without altering their steady-state levels of germline $C_H\alpha$ RNA. In contrast, CD40 ligand or TGFβ alone stimulated an increase in the levels of germline $C_H\alpha$ RNA, and this was associated in each case with an increase in switching to IgA (up to 12%). However, an additive effect on switching to IgA by CD40 ligand plus TGFβ (22%) is observed in the absence of an associated increase in germline $C_H\alpha$ RNA levels above that seen with either agent acting alone. This latter finding suggested the possibility that, in addition to IL-4, CD40 ligand, TGFβ, or both may enhance $S\mu$–$S\alpha$ rearrangement events independently of alterations in germline $C_H\alpha$ transcription. IL-4 also augments switching to IgA in normal murine B cells activated in vitro with LPS or CD40 ligand plus IL-5, anti-Ig-dex, and TGFβ (McIntyre et al., 1995).

IL-5

Dextran-conjugated anti-immunoglobulin antibodies (anti-Ig-dex) stimulate resting B cells to synthesize DNA (Brunswick et al., 1988). In consideration of the accessibility model it was predicted that induction of germline $C_H\gamma1$ transcription in response to the IL-4 IgG1 switch factor would be sufficient to trigger $S\mu$–$S\gamma1$ rearrangement in B cells induced to enter the cell cycle and synthesize DNA upon anti-Ig-dex activation. However, despite IL-4 induction of germline $C_H\gamma1$ RNA in anti-Ig-dex-activated B cells, no corresponding increase in the percentage of $mIgG1^+$ cells or $S\mu$–$S\gamma1$ rearrangement events was observed (Mandler et al., 1993b). Surprisingly, further addition of IL-5, a cytokine initially described for its ability to promote B cell differentiation to immunoglobulin secretion, strongly induced the appearance of $mIgG1^+$ cells and $S\mu$–$S\gamma1$ rearrangements. IL-5 mediated this effect without altering the levels of germline $C_H\gamma1$ RNA and with only a modest enhancement in

DNA synthesis. In the absence of IL-4-mediated targeting of the $C_H\gamma1$ gene, IL-5 by itself fails to induce switching to IgG1 in anti-Ig-dex-activated cells. This suggested that IL-5 provides a critical signal that allows switching to IgG1 to occur in a cycling B cell with a transcriptionally active germline $C_H\gamma1$ gene. Since IL-4 is required for germline $C_H\gamma1$ RNA expression, another independent role(s) for this cytokine in promoting $S\mu$–$S\gamma1$ rearrangement cannot be assessed in this system. IL-5 has also been found to promote switching to IgE, as well as IgG1, without altering the corresponding levels of germline $C_H\epsilon$ and $C_H\gamma1$ RNA in anti-immunoglobulin-activated normal murine B lymphoblasts cultured in the presence of LPS (Purkerson and Isakson, 1992).

IL-10

IL-10 strongly inhibits the generation of $mIgA^+$ cells and $S\mu$–$S\alpha$ rearrangements in normal murine B cells in response to combined activation with LPS, IL-4, IL-5, anti-Ig-dex, and TGFβ (Shparago et al., 1996). This effect of IL-10 occurs in the absence of significant alterations in germline $C_H\alpha$ RNA expression or substantial changes in DNA synthesis. In contrast, IL-10 augments the generation of $mIgG3^+$ cells and $S\mu$–$S\gamma3$ rearrangements in response to activation with LPS alone (Shparago et al., 1996). The inductive effect of IL-10 on LPS-mediated switching to IgG3 is also not associated with significant alterations in germline $C_H\gamma3$ RNA, although IL-10 inhibits LPS-mediated DNA synthesis. Thus, IL-10 selectively regulates immunoglobulin class switching in murine B cells in a manner that appears to be independent of changes in germline C_H transcription. IL-10 also induces switching to IgG1 and IgG3 in CD40-activated naive human B cells (Briere et al., 1994; Malisan et al., 1996), but the mechanism underlying this selectivity has not yet been reported. However, activation of human B cells with anti-CD40 antibody alone induces multiple germline C_H RNAs, including IgG1 and IgG3 (Jumper et al., 1994). This suggests that, as in the mouse, IL-10 may promote switching in human B cells in a manner that is independent of alterations in germline C_H RNA expression. Alternatively, IL-10 may up-regulate the levels of germline $C_H\gamma1$ and $C_H\gamma3$ RNA above some threshold that is critical for switching to occur.

IFNγ

IFNγ promotes switching to IgG2a in LPS- and anti-Ig-dex-activated B cells (Snapper and Paul, 1987; Snapper et al., 1992). These effects of IFNγ are associated with a corresponding induction of germline $C_H\gamma2a$ RNA (Severinson et al., 1990; Collins and Dunnick, 1993). IFNγ also induces switching to IgG3 in anti-Ig-dex-activated B cells and, likewise, this induction correlates with an increase in the levels of germline $C_H\gamma3$ RNA (Snapper et al., 1992). In contrast, IFNγ inhibits LPS-mediated switching to IgG3 and IgG2b (Snapper and Paul, 1987). Whereas inhibition of switching to IgG2b is associated with a reduction in the levels of germline $C_H\gamma2b$ RNA, as anticipated by the accessibility model, IFNγ suppression of LPS-induced switching to IgG3 is not associated with a corresponding reduction in the levels of germline $C_H\gamma3$ RNA (Severinson et al., 1990; Zelazowski et al., 1995). The ability of IFNγ to suppress switching to IgG3 in LPS-activated cells could either result from a direct inhibitory effect of IFNγ on $S\mu$–$S\gamma3$ recombination or perhaps reflect sequential switching from IgM to IgG3 to IgG2a.

Membrane Immunoglobulin Cross-Linking

Multivalent mIg cross-linking, using anti-Ig-dex, by itself induces a modest increase in the levels of germline C_H RNA specific for IgG3, IgG1, and IgG2b in resting B cells (Zelazowski et al., 1995). This is associated with the induction of small amounts of secreted IgG3 and IgG1, although not IgG2b, by anti-Ig-dex-activated B cells in the presence of IL-5. The additional capacity of anti-Ig-dex to inhibit switching to IgE in B cells activated with LPS plus IL-4 is associated with a marked reduction in the levels of germline $C_H\epsilon$ RNA, consistent with the accessibility model (Peçanha et al., 1993; Zelazowski et al., 1995). However, the 50% inhibition of LPS plus IL-4–mediated switching to IgG1 by anti-Ig-dex is associated with a greater than 7-fold increase in the levels of germline $C_H\gamma1$ RNA (Zelazowski et al., 1995). Hence, mIg cross-linking can selectively regulate immunoglobulin class switching in a manner that appears to be both dependent and independent of changes in C_H gene transcription.

p50/NF-κB

The IgH locus contains multiple binding sites for the NF-κB/Rel family of transcription factors. In particular, p50-binding sites have been identified in $I\gamma3$ (Gerondakis et al., 1991), $I\gamma1$ (Lin and Stavnezer, 1996), and $I\epsilon$ (Delphin and Stavnezer, 1995), as well as in 3′E-HS1,2 and 3′E-HS4 (Michaelson et al., 1996). In addition, p50-binding sites have been identified in $S\gamma3$, $S\gamma1$, and $S\gamma2b$ in proximity to sites of recombination within the switch region (Wuerffel et al., 1990, 1992; Kenter et al., 1993). In this regard, B cells from mice made genetically deficient in p50/NF-κB ($p50^{-/-}$) demonstrate selective defects in germline C_H RNA expression and immunoglobulin class switching in vitro relative to wild-type controls (Snapper et al., 1996). In $p50^{-/-}$ B cells, defective expression of $C_H\gamma3$ and $C_H\epsilon$ RNA correlate with a corresponding inability to switch to IgG3 and IgE, as anticipated by the accessibility model. Likewise, normal expression of germline $C_H\gamma1$ RNA by $p50^{-/-}$ B cells is associated with near normal levels of switching to IgG1. However, $p50^{-/-}$ B cells demonstrate a substantial defect in switching to IgA despite expressing normal levels of germline $C_H\alpha$ RNA. These data suggest a role for p50/NF-κB in regulating immunoglobulin class switching that is both dependent and independent of alterations in germline C_H transcription.

A Role for Ku in Switch Recombination?

The nuclear components responsible for effecting the switch recombination event are unknown. Potential candidates may include a variety of regulatory proteins that participate in DNA replication, repair, and/or recombination and whose expression could in theory be regulated. In this regard, a recent report compared the capacity of B cell precursors from RAG-2-deficient and SCID mice to undergo $S\mu$–$S\epsilon$ recombination in response to anti-CD40 monoclonal antibody plus IL-4 (Rolink et al., 1996).

The RAG genes (*RAG-1* and *RAG-2*) are key mediators of VDJ recombination (Mombaerts et al., 1992; Shinkai et al., 1992). SCID mice lack the Ku70/Ku86-associated DNA-dependent serine/threonine protein kinase (DNA-PK; p350), which has also been implicated in VDJ recombination (Bosma et al., 1983; Blunt et al., 1995). B cell precursors from both the RAG-2 and SCID mice expressed germline $C_H\epsilon$ RNA in the presence of anti-CD40 monoclonal antibody plus IL-4 (Rolink et al., 1996). However, RAG-2-deficient, but not SCID, B cell precursors undergo $S\mu$–$S\epsilon$ recombination under these conditions. This suggests that DNA-PK, though not RAG-2, is a key mediator of switch rearrangement. If the level of expression of the functional Ku complex in B cells can be regulated during activation, this would represent one potential pathway for regulating $S\mu$–Sx recombination independent of alterations in germline C_H transcription.

Overall, the nature of the switch-recombinase activity and its targeting factors still remains largely unknown. That at least some of these parameters may be regulated within the B cell is suggested by the capacity of various cytokines, B cell activators, and transcription factors to effect changes in switch recombination without altering the expression of the corresponding germline C_H genes. These model systems should help to elucidate the nature of these factors.

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